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Genetic and Hematological Studies in a Group of 114 Adult Patients With SC Sickle Cell Disease

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The clinical and biological heterogeneity of sickle cell hemoglobin (Hb) C disease (SC disease) is similar to sickle cell anemia, but has a much milder course. The effect of genetic factors such as α thalassemia or β-globin gene haplotype has been analyzed in a limited number of cases. In this work, we report about 114 adult SC patients, aged 15 to 65 years (M/F = 0.93). The frequency of deletional α thalassemia ($\alpha^{-3.7}$) was found to be about 35%. The coinheritance of an α-thalassemia trait with SC disease had no effect on the hemoglobin level but hemolysis was significantly reduced. In these patients, as described for homozygous Hb S individuals, the Hb F level was higher in females than in males and in individuals carrying the β^{s}-Senegal haplotype. This haplotype involves the presence of an XmnI site 5′ to Gγ, which is considered responsible for an increased Gγ/Aγ ratio. Our survey showed that some genetic factors may modulate hematological parameters in SC disease. Am. J. Hematol. 59:15–21, 1998.

Key words: SC sickle cell disease; alpha thalassemia; beta-globin gene haplotypes

INTRODUCTION

Approximately 25% of the patients followed in the Sickle Cell Center of Hôpital Henri Mondor (Créteil, France) are compound heterozygous for hemoglobin (Hb) S and Hb C. Their clinical conditions differ from those of individuals who are homozygous for Hb S. They have less frequent painful crises and chronic organ damage but present a particular incidence of retinopathy, aseptic necrosis of the head of long bones, and pathological events involving the spleen during adulthood. These patients display a wide spectrum of clinical severity, analogous to that observed in sickle cell anemia [1].

The SC patients studied here were followed in our center for several years. They originate either from North Africa, West Africa, or the West Indies, are the first generation of migrants, and have similar socioeconomic backgrounds.

The hematological data, hemolysis parameters, and Hb F levels of these patients were studied in the steady state. The genotype/phenotype relationships were determined, by correlating α thalassemia and β-globin gene haplotypes with the biological data.

MATERIALS AND METHODS

Patients

We excluded from the study patients who were under hydroxyurea treatment, those who suffered from painful crises in the previous two months, those who were transfused in the previous three months, and pregnant women. One hundred fourteen patients with a mean age of 32.5 years (range 15–65 years; M/F 0.93) fulfilled these criteria. They were of various geographical origins: Sixty-seven originated from the Caribbean, 41 from West Africa (including one from Cameroon), and six from North Africa.

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TABLE I. Hematological and Biochemical Parameters of 114 Hb SC adults (mean value ± SD)†

	Total n = 114	Males n = 55	Females n = 59	*P**
Hb F (%)	1.63 ± 1.20	1.24 ± 0.89 (<1)	1.97 ± 1.33 (<1)	0.001
RBC (10^{12}/L)	4.43 ± 0.76	4.73 ± 0.91 (5.5 ± 1)	4.19 ± 0.49 (4.8 ± 1)	<0.001
Hb (g/dL)	11.8 ± 1.4	12.3 ± 1.7 (13–17.7)	11.3 ± 0.95 (11.5–16)	<0.001
MCV (fL)	81.6 ± 7.9	81.2 ± 9.8 (80–100)	82.0 ± 5.8 (80–100)	0.43
MCH (Pg)	27.0 ± 2.9	26.8 ± 3.7 (27–32)	27.1 ± 2.0 (27–32)	0.37
Retic (10^9/L)	113 ± 59	117 ± 57 (25–100)	109 ± 61 (25–100)	0.34
WBC (10^9/L)	8.0 ± 3.0	8.1 ± 2.8 (4–10)	7.8 ± 3.1 (4–10)	0.39
PMN (10^9/L)	4.3 ± 2.0	4.3 ± 1.8 (1.8–7.5)	4.3 ± 2.3 (1.8–7.5)	0.65
Lymphocytes (10^9/L)	2.7 ± 1.1	2.8 ± 1.1 (1.0–4.0)	2.7 ± 1.0 (1.0–4.0)	0.72
Platelets (10^9/L)	237 ± 107	256 ± 116 (150–500)	277 ± 99 (150–500)	0.13
FB (μMol/L)	20 ± 14	24 ± 17 (<13)	17 ± 11 (<13)	0.004
LDH (UI/L)	219 ± 73	233 ± 89 (100–250)	206 ± 53 (100–250)	0.08
Creatinine (μMol/L)	93 ± 114	118 ± 165 (60–130)	71 ± 15 (60–130)	<0.001**
Uric acid (μMol/L)	330 ± 113	377 ± 135 (145–460)	289 ± 68 (145–460)	<0.001**
Ferritin (μg/L)	186 ± 184	186 ± 217 (80–250)	186 ± 151 (50–120)	0.40**

†Hb, hemoglobin; SC, sickle cell hemoglobin C; SD, standard deviation; RBC, red blood count; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; WBC, white blood cell; PMN, polymorphonuclear neutrophil leukocytes; FB, free-bilirubin; LDH, lactic dehydrogenase. Normal values of the laboratory are given within brackets.

**P* is the degree of significance comparing male and female patients.

**Skewed distribution.

Biological and Molecular Investigations

Phenotypes were identified by isoelectrofocalization, citrate agar electrophoresis of the lysate, and Hb solubility test.

Hb F was measured by cation exchange high-performance liquid chromatography (VARIANT Biorad, Hercules, CA) using the β-Thal short program. The Gγ/Aγ composition of Hb F was determined in 20 patients using reversed-phase perfusion chromatography [2].

Hematological data were obtained by standard methods: Blood cell counts and red blood cell (RBC) indices were measured on a Coulter®-STKS (Coulter Co., Miami, FL); reticulocyte count by flow cytometry on a Coulter®-Profile 1.

Total bilirubin and conjugated bilirubin were measured by colorimetry, serum lactic-dehydrogenase (LDH) by enzymatic method and serum ferritin by immunoenzymatic assay. Other biochemical parameters such as serum creatinine and serum uric acid were obtained on a Dax®-Analyzer (Technicon Instruments Co., Tarrytown, NY) by colorimetry.

DNA were isolated from peripheral blood leukocytes by phenol-chloroform extraction. Three forms of deletional α thalassemia ($\alpha^{-3.7}$, $\alpha^{-20.5}$, and Mediterranean type) were ascertained by polymerase chain reaction (PCR) method [3,4].

PCR-restriction fragment length polymorphism (RFLP) were used to determine the β-globin gene cluster haplotypes [5]. The polymorphic restriction endonuclease sites studied were: HincII 5′ to the ϵ- and 3′ to the ψβ-globin genes, XmnI (−158) 5′ to the Gγ-globin gene, HindIII in the IVS-2 of Gγ-and Aγ-globin genes, HinfI 5′ and 3′ to the β-globin gene and RsaI 5′ to the β-globin gene.

Statistical Analysis

Statistical significance was calculated using the nonparametric tests of Kruskal-Wallis and Mann-Whitney.

RESULTS

Biological Parameters

As shown in Table I, a mild anemia was observed. Mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH) were found at the lower limit of the normal range, whereas mean corpuscular hemoglobin concentration (MCHC) (not given in the table) was normal. We must nevertheless point out that these measurements were performed on a machine that calculates the RBC indices from the measured values of cell volume. By this method, it has been shown that dehydrated and less deformable RBCs, like SC cells, may result in a erroneously high level of MCV and, consequently, an erroneously low value for MCHC [6–8]

The RBC counts and the Hb levels were both significantly higher in males than in females. The other data indicated a moderate hemolytic process because bilirubin, lactate dehydrogenase (LDH) and reticulocyte counts were normal or slightly elevated. These last two parameters were identical in males and females whereas free-bilirubin was significantly higher in males than in

TABLE II. Hematological and Biochemical Characteristics of Hb SC Adults With and Without α Thalassemia (Mean Value ± SD)†

	Non α thalassemia n = 63 (M/F, 0.70)	α thalassemia n = 35 (M/F, 1.18)	*P**
Hb F (%)	1.61 ± 1.18	1.66 ± 1.31	0.92
RBC (10^{12}/L)	4.23 ± 0.58	4.78 ± 0.92	<0.001
Hb (g/dL)	11.8 ± 1.2	11.9 ± 1.8	0.33
MCV (fL)	84.4 ± 7.5	76.7 ± 6.8	<0.001
MCH (Pg)	28.0 ± 2.7	25.2 ± 2.5	<0.001
Retic (10^9/L)	125 ± 67	95 ± 39	0.06
WBC (10^9/L)	8.4 ± 3.0	7.1 ± 3.1	0.017
PMN (10^9/L)	4.5 ± 2.0	3.7 ± 2.1	0.013
Lymphocytes (10^9/L)	3.0 ± 1.1	2.5 ± 0.9	0.052
Platelets (10^9/L)	290 ± 94	222 ± 93	0.001
FB (μMol/L)	20 ± 14	19 ± 15	0.13
LDH (UI/L)	233 ± 77	182 ± 44	<0.001
Creatinine (μMol/L)	90 ± 115	100 ± 124	0.15**
Uric acid (μMol/L)	316 ± 80	348 ± 155	0.37**
Ferritin (μg/L)	220 ± 169	142 ± 204	<0.001**

†See Table I footnote.
**P* is the degree of significance comparing the adult SC patients with and without α thalassemia.
**Skewed distribution.

females. White cells and platelet counts did not differ from normal controls.

A skewed distribution of the population in relation with iron overload is responsible for the large standard deviation (SD) values reported for ferritin. The distributions of serum creatinine and uric acid values were also biased because three patients suffered from chronic renal failure; two of them were under regular hemodialysis.

Hb F ranged from 0.1% to 6.5%, with a mean value of 1.63%. This level was significantly higher in females than in males ($P = 0.001$).

Alpha Gene Status

The α-globin gene status could only be determined in 105 of the 114 patients.

The only form of deletional α thalassemia that we observed was the −3.7 Kb type. It was found in 35.2% of the individuals; 34 were heterozygous (−α/αα) and three were homozygous (−α/−α). It affected 34.4% of the Caribbean group and 41.4% of the West African group, but was not found in the small group of North African patients.

As shown in Table II, the coinheritance of the $\alpha^{-3.7}$ deletion with Hb SC disease significantly reduced MCV and MCH whereas erythrocytes, white blood cells (WBCs) and platelets were increased. Mean LDH activity was reduced but free-bilirubin concentration was not modified. Alpha thalassemia did not influence either the total Hb level or the Hb F levels.

β-Globin Gene Cluster Haplotypes

The haplotype of the 228 chromosomes was determined (Table III).

Except for eight atypical cases, shown in Figure 1, the β^s mutation was associated with one of the four major African haplotypes. In 66.6% of the cases, the β^s chromosome was linked to the Benin haplotype, the only one observed in the patients from the North Africa group. The Bantu and the Cameroon haplotypes were only found in the Caribbean group (Table IV).

Two haplotypes were found to be associated with the β^c mutation—the common type designated as β^cI and the less frequent one, β^cII. These haplotypes were initially described by Boehm et al. [9] and also were reported in other surveys concerning Black Americans [10–13]. We found the β^cII haplotype in individuals originating from all three of the ethnic groups (Table IV).

The biological parameters of individuals carrying the β^cI or β^cII haplotypes were similar (data not shown). The difference in Hb F levels between both groups (1.62% and 2.32%) was not statistically significant ($P = 0.21$).

When the hematological parameters were analyzed according to the β^s haplotypes, significant differences appeared (Table V). The mean Hb F levels were higher in patients with the β^s Senegal haplotype than in the other groups.

Hb F composition was determined in five patients carrying the β^s Senegal haplotype; the percentage of the Gγ chains ranged from 56% to 75% with a mean value of 63%. Patient n°8 in Figure 1, who displayed an atypical haplotype, but with a Senegal 5′ subhaplotype (XmnI site 5′ Gγ), a Gγ of 61% was found, together with a low Hb F level (0.8%). The mean Gγ was 38% in the other patients. This value is the same as that usually observed in normal adults [14].

TABLE III. The β-Globin Gene Cluster Haplotypes: Distribution in the 114 Hb SC Patients*

	β^s haplotypes					
β^c haplotypes	Benin	Bantu	Cameroon	Senegal	Undetermined	Total
Typical (β^cI)	70 (61.4%)	15 (13.1%)	3 (2.6%)	10 (8.7%)	0	98 (85.9%)
Type II (β^cII)	6 (5.2%)	1 (0.8%)	0	1 (0.8%)	0	8 (7.0%)
Undetermined	0	0	0	0	8 (7.0%)	8 (7.0%)
Total	76 (66.6%)	16 (14.0%)	3 (2.6%)	11 (9.6%)	8 (7.0%)	114

*Hb, hemoglobin; SC, sickle cell hemoglobin C.

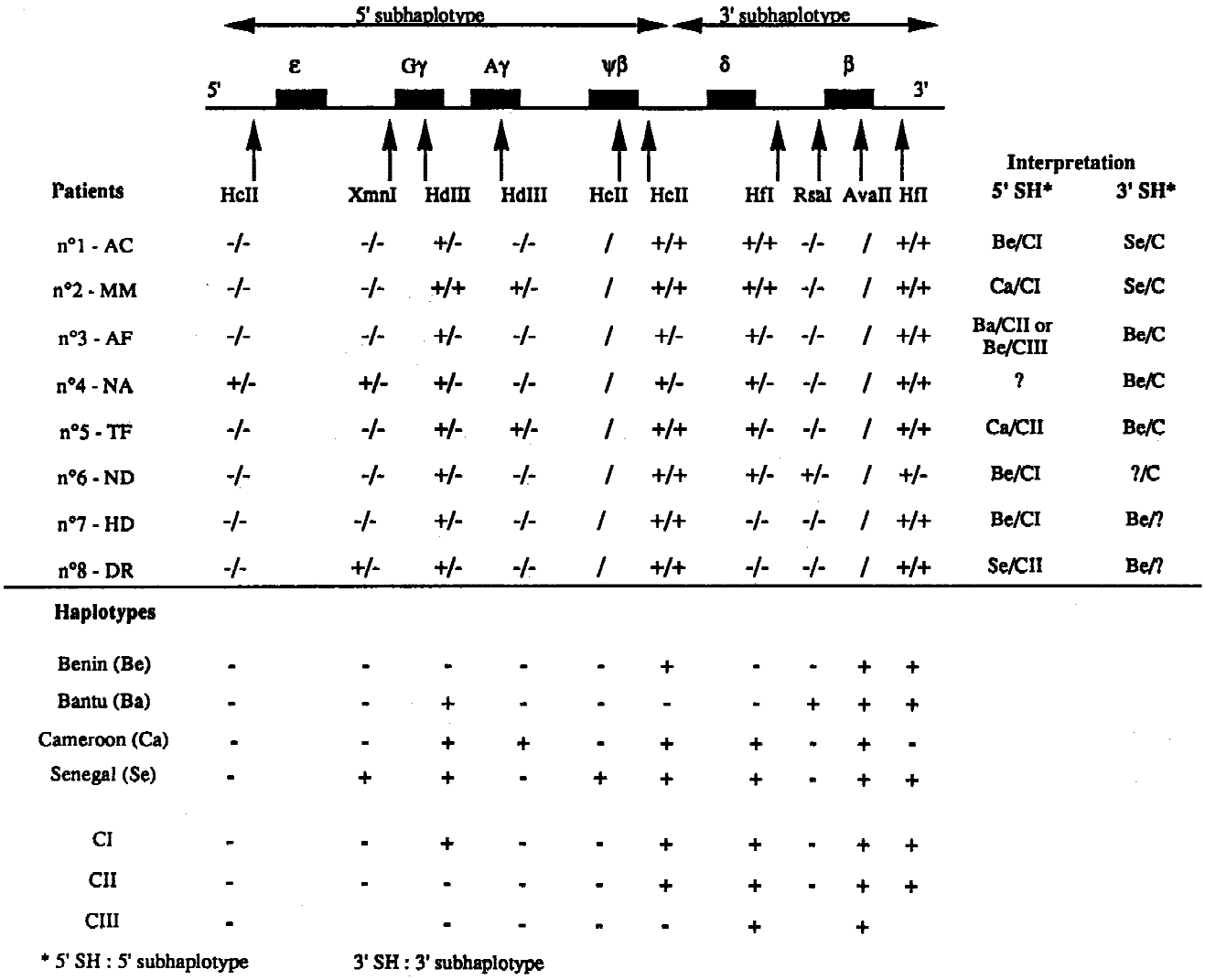

Fig. 1. Undetermined β-globin gene haplotypes in eight SC patients.

DISCUSSION

Because of its moderate clinical course, SC disease has been given limited study on a limited population of patients. However, the clinical severity of SC disease is heterogeneous and, as for homozygous sickle cell disease, genetic factors, coinherited with the sickle mutation, have to be tested for their possible modulating effects.

Steinberg et al. [15] studied 53 adults with SC disease and found no relationship between α-globin genotype and hematocrit, frequency of painful crises, or organ failure. Rodgers et al. [16], in a case report, attributed a tendency toward a prolonged survival and a milder clinical course to α thalassemia.

Powars et al. [17] in a study of 41 patients with SC disease concluded that there is deleterious influence of

TABLE IV. Geographical Origin and β-Globin Gene Cluster Haplotypes in 114 Hb SC Patients*

	Ben-CI[a]	Ben-CII[b]	Ban[c]	Cam[d]	Sen[e]	UD[f]
North Africa (n = 6)	3 (50%)	1 (16.6%)	0	0	0	2 (33.3%)
West Africa (n = 41)	27 (65.8%)	3 (7.3%)	0	0	6 (14.6%)	5 (12.1%)
Caribbean[g] (n = 67)	40 (59.7%)	2 (2.9%)	16 (23.8%)	3 (4.4%)	5 (7.4%)	1 (1.4%)

*Hb, hemoglobin; SC, sickle cell hemoglobin C.
[a]β^s Benin haplotype associated with the β^c I haplotype (CI).
[b]β^s Benin haplotype associated with the β^c II haplotype (CII) described by Boehm et al. [9].
[c]β^s Bantu haplotype.
[d]β^s Cameroon haplotype.
[e]β^s Senegal haplotype.
[f]Undetermined haplotypes.
[g]The Caribbean group included patients from the French West Indies, French Guiana, and Hait.

TABLE V. β^s-Globin Gene Haplotypes and Biological Data in Hb SC Adults (Mean Value ± SD)†

	BENIN (n = 76) (M/F, 0.85)	BANTU (n = 19) (M/F, 0.72)	SENEGAL (n = 10) (M/F, 0.66)	*P**
Hb F (%)	1.53 ± 1.18	1.47 ± 0.67	3.24 ± 1.46	0.003
RBC (10^{12}/L)	4.46 ± 0.75	4.22 ± 0.69	4.47 ± 0.60	NS
Hb (g/dL)	11.9 ± 1.3	11.3 ± 2.0	11.8 ± 1.0	NS
MCV (fL)	81.4 ± 8.7	82.8 ± 5.9	80.6 ± 6.6	NS
MCH (Pg)	26.9 ± 3.2	27.8 ± 2.3	26.6 ± 2.2	NS
Retic (10^9/L)	116 ± 59	102 ± 59	102 ± 63	NS
WBC (10^9/L)	7.7 ± 2.7	7.7 ± 2.7	10.0 ± 5.4	NS
PMN (10^9/L)	4.1 ± 1.7	4.3 ± 2.1	5.8 ± 3.8	NS
Lymphocytes (10^9/L)	2.7 ± 1.1	2.3 ± 0.8	3.2 ± 1.2	NS
Platelets (10^9/L)	257 ± 99	295 ± 133	252 ± 113	NS
FB (μMol/L)	22 ± 17	17 ± 8	13 ± 14	NS
LDH (UI/L)	218 ± 79	222 ± 67	206 ± 28	NS
Creatinine (μMol/L)	78 ± 15	73 ± 12	73 ± 14	NS
Uric acid (μMol/L)	326 ± 87	268 ± 51	343 ± 75	0.03
Ferritin (μg/L)	182 ± 156	131 ± 123	173 ± 200	NS

†See Table I. NS, not significant.
**P* is the degree of significance comparing the adult SC patients according to their β^s-globin gene haplotypes by the Kruskal-Wallis test. Hb F appears with a level of significance at 0.003. The comparison of the three haplotype groups two by two using the Mann-Whitney test showed that this significant difference was due to the β^s-Senegal haplotype. Hb F Benin/Senegal, $P = 0.001$; Hb F Bantu/Senegal, $P = 0.002$, Hb F Benin/Bantu, $P = 0.45$.

the β^s Bantu haplotype. These authors found that patients who had the β^s Bantu haplotype had lower Hb concentrations, a higher frequency of chronic renal failure, retinopathy, and priapism than SC patients who had other β^s haplotypes. In a recent work, Steinberg et al. [13] observed no effect of β-globin gene haplotype on hematological features in 73 adult patients with SC disease.

In our study, Hb F ranged from 0.1% to 6.5% (mean value ± SD; 1.63% ± 1.20%). Similar values were found by Schroeder et al. [10] among 41 SC patients. In contrast, Ballas et al. [18] and Labie et al. [19] reported lower Hb F levels, less than 2% and 1.5%, respectively. This could be explained by the presence of individuals with a β^s Senegal haplotype in our group. Nevertheless, in Table I, the significant difference in Hb F levels between males and females is very likely due to gender since patients having the β^s Senegal haplotype were equally distributed. This significantly higher level of Hb F in females than in males, has already been described in AA individuals and SS patients, and is related to a possible role of some X-chromosome polymorphisms in the regulation of the γ-globin gene expression [20].

Alpha thalassemia was found with high frequency, similar to that observed in individuals of African descent,

with or without sickle cell disease [10,21,22]. In agreement with Steinberg et al. [13,15], α thalassemia showed no effect on the Hb level. However, our study suggests that α thalassemia may reduce hemolysis in SC disease, compared to that observed in SS patients [23,24]. The higher reticulocyte, leukocyte, and platelet counts found in patients without α thalassemia (αα/αα) than in those with an α thalassemia (−α/αα or −α/−α) may result from a compensating hematopoietic response to an increased hemolytic activity. The differences observed in the RBC indices (MCV and MCH) between patients with and without α thalassemia were found to be statistically related to the α gene status, but not to gender, whereas RBC count and LDH mean value were both significantly but independently correlated with these two characteristics.

The β^s chromosome haplotypes differed depending on the geographical origin of the patients. The four major African β^s haplotypes [25,26] were found in the Caribbean patients of our group, as reported in most of the studies on African Americans with SC disease [10,11]. The small differences in the frequencies of each β^s haplotype observed in these various surveys probably reflect the origin of the slave traffic from Africa to America. In the African patients, with the exception of a few Senegal haplotypes in individuals originating from West Africa, the Benin type was the main β^s haplotype observed. It is not surprising because we know that in West African and North African countries, the Benin haplotype is predominant [25].

There is a general agreement for a unicentric origin of the β^c mutation. This mutation arose on an uncommon β^A chromosome in Central West Africa, leading to the β^cI haplotype. It was then followed by a concentric and decreasing diffusion. Other haplotypes are rare and probably occurred by recombination [9,11–13]. Labie et al. [19], studying an homogeneous SC population who originated from Burkina Faso and Benin found uniformly the β^s Benin haplotype associated with the common β^c haplotype. The second β^c linked haplotype (β^cII), initially described by Boehm et al. [9] could have arisen by recombination between the Benin 5′ subhaplotype and the typical β^c 3′ subhaplotype [12,19]. The present survey showed a wide geographical distribution of this β^cII haplotype since it was found in our three groups of patients. One hypothesis is that the recombination occurred before the diffusion of this β^c bearing chromosome. Studies on β^c chromosomes [9–13,19] in African Americans, North Africans, or West Africans have reported the presence of the β^cII haplotype but it has never been described in Caribbean individuals. Kéclard et al. [22] found the common β^cI haplotype in all the 26 SC patients from Guadeloupe. In our study, three of the 67 Caribbean patients (4.5%) were bearing a β^cII chromosome.

Atypical haplotypes (called ''undetermined'' in Tables III and IV, and Figure 1) were found in 7% of the cases and distributed in each ethnic group. Such high frequency of undetermined haplotypes was reported among 73 SC patients studied by Steinberg et al. [13]. The difficulty in characterizing these haplotypes using the PCR-RFLP method was essentially attributed to the fact that SC disease is a compound heterozygous state. It would be of interest to study families in order to determine if these haplotypes result from recombination or mutation at one or more restriction site.

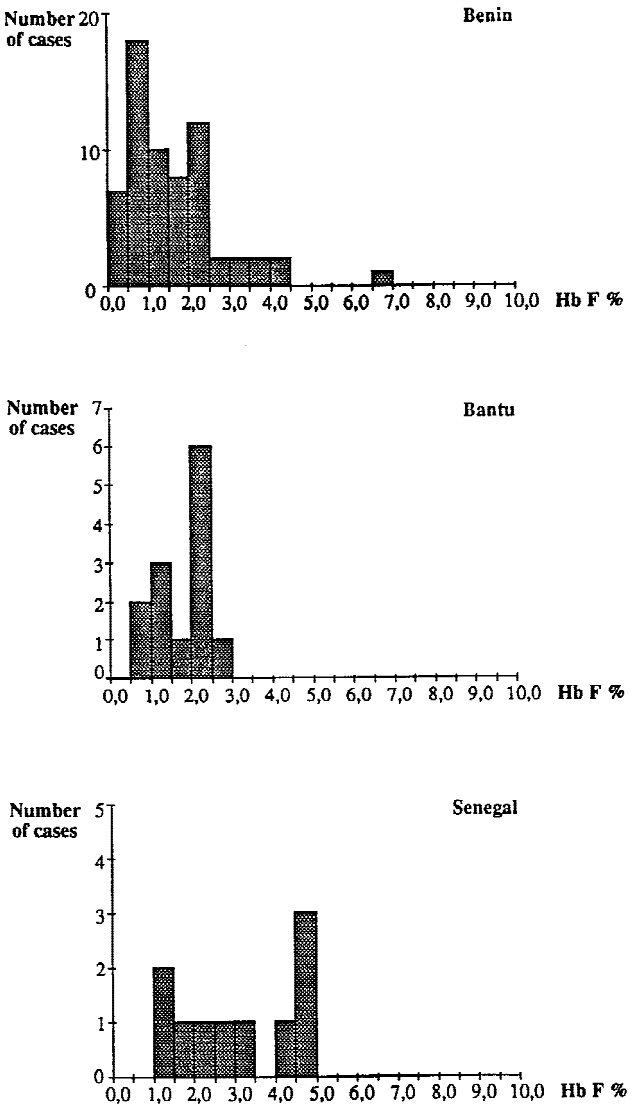

Fig. 2. Distribution of Hb F (%) in Hb SC adults according to the β^s-globin gene haplotypes.

Steinberg et al. [13] found no influence of the β^s haplotype on the hematological characteristics or on the Hb F levels, whereas Powars et al. [17] reported low Hb concentrations in individuals with SC disease who were carrying a β^s Bantu chromosome. In our group of patients, Hb F levels appeared strongly associated with the β^s Senegal haplotype (Figure 2). Statistical analysis

showed that this result was not influenced by the α gene status. In addition, within each haplotype, when gender was taken into account, a higher level of Hb F was found in females than in males. But when considering only the males or the females, the level of Hb F was significantly linked to the haplotype.

We also observed a high correlation between the presence of the XmnI site 5′ to the Gγ gene and an increased level of Gγ chains. Such findings have been reported previously in SS patients [23,25]. Several authors [22,27] only reported the association of the positive XmnI site 5′ to Gγ with a high expression of Gγ chains even though Hb F levels were not increased. Such a discrepancy from one survey to another suggests a role of other factors, genetically determined or not, in Hb F expression.

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